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Seasonal Effects on Microbial Transformation Rates of an Herbicide in a Freshwater Stream Application of Laboratory Data to a Field Site

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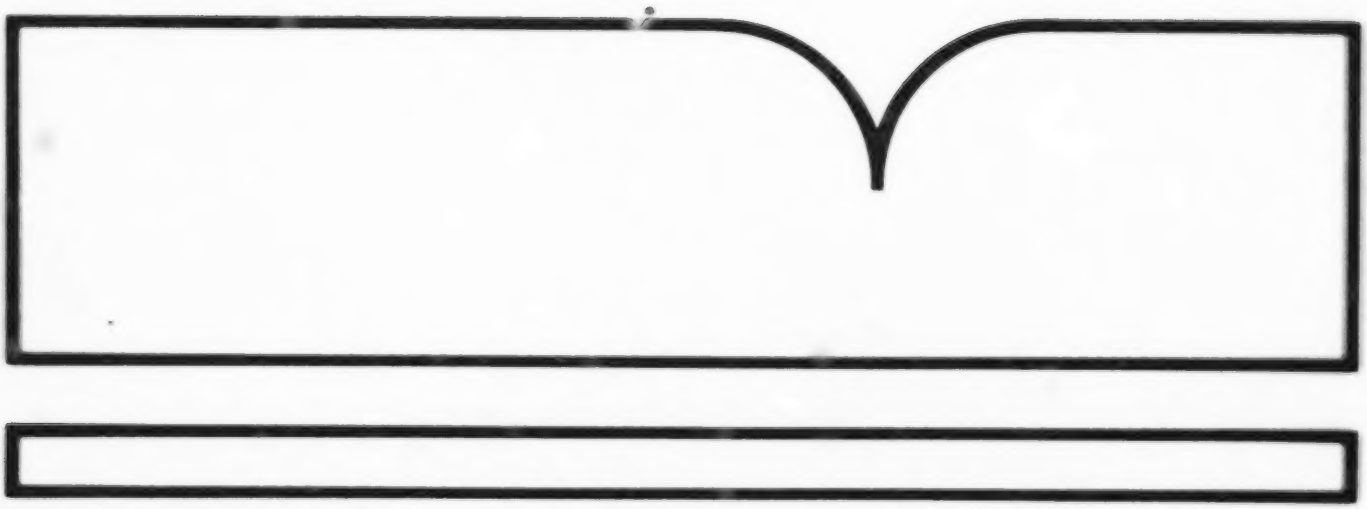

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16. ABSTRACT

Seasonal effects on microbial transformation rates of an herbicide, 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE), in a freshwater stream were investigated using low concentrations (less than 100 ug L^{-1}) of the herbicide mixed with an inert dye, rhodamine WT, to correct for dilution. Although water temperatures decreased from 22±0.5 to 8.0±0.5 °C and water velocities decreased from 0.09±0.01 to 0.03±0.003 m s^{-1}, microbial transformation rates, as indicated by psuedo-first-order transformation rate coefficients, increased as much as three-fold in the cold months over those in the spring and summer. The increased winter transformation rates were associated with leaf-litter accumulation which provided additional colonized surface area in the stream. Therefore, the increased colonized surface area more than compensated for the effects of temperature and water velocity (under conditions of mass transport limitation) that otherwise would have reduced microbial transformation rates in the winter months.

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SEASONAL EFFECTS ON MICROBIAL TRANSFORMATION RATES OF AN HERBICIDE IN A FRESHWATER STREAM: APPLICATION OF LABORATORY DATA TO A FIELD SITE

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Abstract—Seasonal effects on microbial transformation rates of an herbicide, 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE), in a freshwater stream were investigated using low concentrations (less than 100 $\mu g\ L^{-1}$) of the herbicide mixed with an inert dye, rhodamine WT, to correct for dilution. Although water temperatures decreased from 22 ± 0.5 to 8.0 ± 0.5°C and water velocities decreased from 0.09 ± 0.01 to 0.03 ± 0.003 m s^{-1}, microbial transformation rates, as indicated by pseudo-first-order transformation rate coefficients, increased as much as threefold in the cold months over those in the spring and summer. The increased winter transformation rates were associated with leaf-litter accumulation, which provided additional colonized surface area in the stream. Therefore, the increased colonized surface area more than compensated for the effects of temperature and water velocity (under conditions of mass-transport limitation) that otherwise would have reduced microbial transformation rates in the winter months.

Keywords—Herbicides Microbial transformation Biodegradation Seasonal effects Temperature effects

INTRODUCTION

Most of the information available on microbial transformation rates of environmental pollutants has been obtained under controlled laboratory conditions designed to aid in the formulation of kinetic models appropriate for describing pollutant transformation rates and to evaluate the effects of various parameters on those rates. Because of the uncontrollability, complexity, inconvenience, manpower requirements and expense of field studies, very few investigators have studied microbial transformation rates in the field, particularly with regard to aquatic ecosystems. Field studies, however, are valuable for evaluating the applicability of laboratory-derived models to field situations. They are useful for testing the completeness of the models, i.e., that they include and describe the significant environmental processes that affect pollutants. Whenever the observed behavior of a pollutant significantly deviates from what was predicted using a mathematical model, it is evident that one or more significant environmental processes was neglected or improperly described.

Microbial transformation rates may be influenced by such diverse environmental factors that the applicability of laboratory-derived models to field conditions is a matter meriting investigation. Therefore, having evaluated the effects of pH, water turbulence and substrate concentration on microbial transformation rates of an herbicide, 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE), under laboratory conditions [1,2], we decided to continuously spike a field stream with the compound over a 9-month period to determine whether our knowledge of the effects of various environmental factors was sufficient to account for any observed variations of microbial transformation rates at the field site during different seasons.

MATERIALS AND METHODS

Site description

Field experiments were done at Chalet Stream, a small freshwater stream in Whitehall Forest,

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Clarke County, Georgia. The stream was approximately 1 m wide and 10 to 30 cm deep, located in a densely wooded area predominantly populated with deciduous trees. The average water velocity was 0.07 m s^{-1} (SD = ±0.034; n = 6) during the study, as determined from rates of dye movement in the stream. Although there were no sudden drops in elevation of the streambed, there were numerous crooks and turns so that the slug of dye and test chemical was well mixed over a vertical cross-section as it moved downstream. Sampling Stations 1, 2 and 3 were established, respectively, at points located 11.3, 91.1 and 153.6 m downstream from the point at which we introduced the test chemicals.

Field procedure

Microbial transformation rate experiments were carried out at various times from June 1984 through February 1985 (Figs. 1 and 2) and in February 1986 (Fig. 3). The stream was spiked with a mixed aqueous stock of 2,4-DBE and rhodamine WT so that the initial stream concentrations were approximately 80 μg L^{-1} for the herbicide and approximately 4 mg L^{-1} for the dye. Rhodamine WT was used to trace the dilution of the herbicide because it has been shown that adsorption of this dye to suspended sediment at concentrations not exceeding 1 g L^{-1} is insignificant [3].

Triplicate water samples were taken from each station as the slug of dye and herbicide moved down the stream. After the Station 3 sample was taken on each sampling date, the stream was spiked a second time for a duplicate rate study. Because of rapid microbial transformation, the herbicide could not be detected at Station 3 in the fall and winter; therefore, Stations 1 and 2 provided the only data for sampling times during cooler months. The water samples were immediately extracted at the site with 2,2,4-trimethyl pentane. The 2,4-DBE extraction efficiency was 101% (SD = ±6.0%; n = 54). Pseudo-first-order microbial transformation rate coefficients, k_1, were determined from ratios of herbicide to dye concentration, using a procedure described below.

On each sampling date, batch samples of water

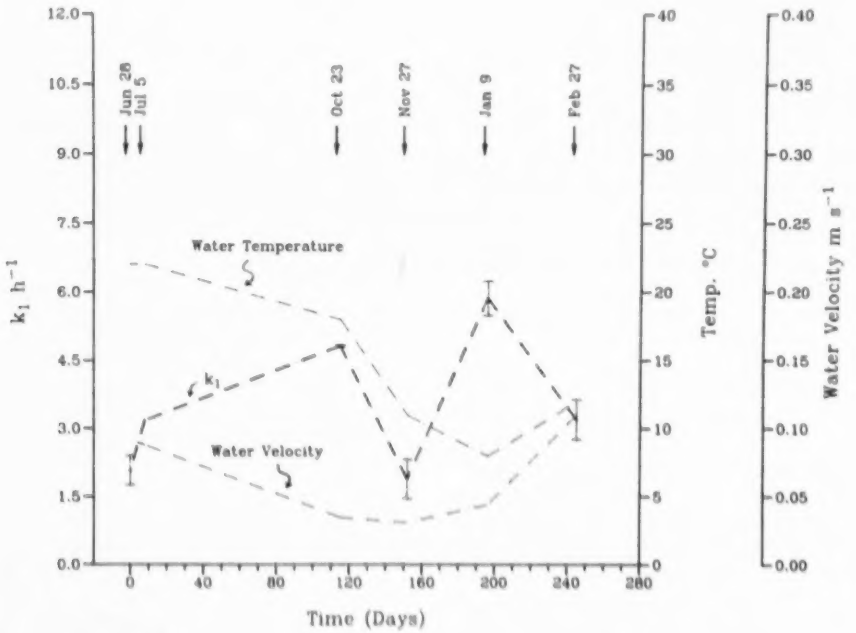

Fig. 1. Seasonal variations of water velocity, temperature and pseudo-first-order microbial transformation rate coefficients, k_1, at the field site. Rate coefficients represent microbial transformation mediated by organisms associated with submerged surfaces after subtracting water column values of k_1. Water column values of k_1 accounted for less than 5% of the microbial transformation. Error bars represent 1 SD of duplicate trials, and k_1 values have been multiplied by −1. Only one trial was conducted on 5 July.

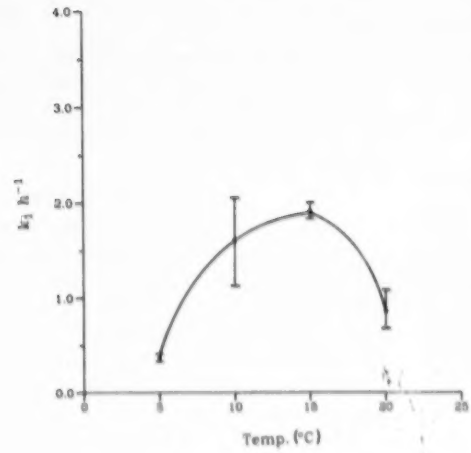

Fig. 2. Effect of temperature on samples of winter-adapted microbiota taken from the field site in February. Data are normalized to microbial transformation rates in batch cultures at 15°C. Error bars represent 1 SE of the mean of duplicate batch cultures, and k_1 values have been multiplied by −1.

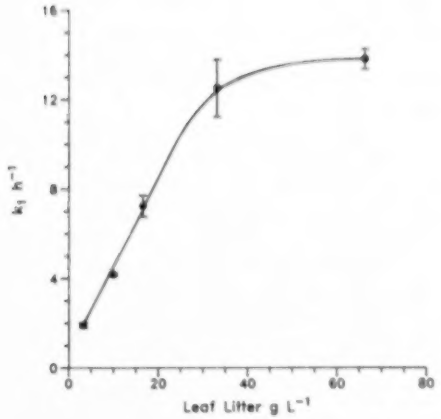

Fig. 3. Relationship between microbial transformation rates of 2,4-DBE and leaf-litter concentrations. Values of k_1 have been multiplied by −1, and the k_1 value from suspended organisms (0.33 ± 0.01 h^{-1}) has been subtracted from each total k_1 value (suspended plus leaf-litter associated organisms). Error bars represent standard error of regression.

were collected at the streamspiking station and taken to the laboratory to determine k_1 values, using a procedure described below, for the water column. These data were used to establish the relative contribution of water column and surface-associated organisms to the transformation process. Also collected were sediment and leaf litter samples for sorption and transformation rate studies performed in the laboratory.

Laboratory experiments

Values of k_1 for the water column were determined by spiking 100-ml samples to approximately 100 μg L^{-1} with 2,4-DBE and measuring the loss of the herbicide over time. These rate studies lasted several hours. The samples were incubated on a shaker (150 rpm) at 20°C. Water samples collected during the winter were tested at temperatures approximating field conditions (about 10°C).

Temperature effects on winter-adapted microbiota were tested by collecting 100-ml duplicate batch cultures of a mixture of water column, sediment and leaf-litter samples, spiking the cultures to approximately 100 μg L^{-1} with 2,4-DBE and measuring the rate of loss of the compound over time in shaker incubators (150 rpm) at 5, 10, 15 and 20°C. These rate studies lasted several hours. Because studies could not be done on replicate samples at all temperatures simultaneously, microbial transformation rates were normalized to rates measured at 15°C (Fig. 2).

The microbial transformation activity of organisms associated with leaf litter (Fig. 3) was tested by spiking 100-ml batch samples to 50 to 90 μg L^{-1} 2,4-DBE and measuring the loss of the herbicide over time. These rate studies lasted for less than one-half hour. Batch samples were collected from the field site and the mass of blotted litter determined on an electronic balance.

Estimates of leaf-litter concentrations at the field site were made in February 1986 by collecting all of the litter in 30 × 30 cm areas and measuring water depth. Five areas were chosen and the mass of blotted litter samples determined on an

electronic balance. Litter coverage of the stream bottom appeared to vary severalfold from one area to another; therefore, litter concentrations at the site represented a gross estimate. Litter-collection sites were selected on the basis of their apparent representativeness of the whole stream.

Sorption of rhodamine WT was tested by spiking five replicate flasks of sediment and five replicate flasks of leaf litter to approximately 0.5 mg L^{-1} dye. The flasks contained 35 g L^{-1} sediment or litter (wet weight) in water collected at the field site. Using water blanks spiked with the same initial dye concentration for comparison, sorption was measured by loss of dye from solution after 1 h in flasks containing sediment or litter. Also, dye samples were taken immediately after spiking the flasks with dye and comparing those concentrations with 1-h samples. These two approaches were used to determine if sorption was so rapid that accurate 0-h samples could not be taken. Both sterile (autoclaved for 1 h) and nonsterile samples were tested in separate experiments. Sorption of 2,4-DBE to autoclaved samples of sediment and leaf litter was tested in a manner similar to that used in experiments with the dye. Sorption to nonsterile samples was not tested; however, previous studies [1] have been done using viable microbiota.

Photodegradation of rhodamine WT was tested by spiking five replicate samples of site water to approximately 0.5 g L^{-1} dye and placing the samples in bright sunlight (at around noon) for 1 h. Open glass beakers were used as containers to allow direct exposure of the solutions. Dye concentrations were determined before and after exposure to sunlight.

Analytical techniques and data processing

2,4-DBE concentrations in 2,2,4-trimethyl pentane extracts were determined using a Tracor 550 gas-liquid chromatograph equipped with a ^{63}Ni detector and a 2-m column (internal diameter, 4 mm) packed with 3% OV-1 on 80/100 mesh Supelcoport. Dye concentrations were determined in the aqueous phase of the extracts using a Varian Cary 219 spectrophotometer.

Pseudo–first-order microbial transformation rate coefficients, k_1, were determined for the field site based on least-squares analyses of plots of the logarithms of 2,4-DBE/dye concentration ratios versus time. Values of k_1 for batch cultures were similarly determined using logarithms of 2,4-DBE concentrations versus time. For both procedures, time refers to the time lapsed after the field site or batch culture was spiked.

Reagents

2,4-DBE (99.8% pure) was supplied by Union Carbide Corporation. Rhodamine WT was supplied by the Environmental Services Division, Region IV, U.S. Environmental Protection Agency, Athens, Georgia.

RESULTS AND DISCUSSION

During the course of the 2,4-DBE transformation rate studies, water temperatures at the field site decreased from a high of 22 ± 0.5°C in June and July to a low of 8.0 ± 0.5°C in January. Water velocities decreased from a high of 0.09 ± 0.01 m s^{-1} in June to a low of 0.03 ± 0.003 m s^{-1} in November. pH, however, remained at a relatively constant 6.6 ± 0.08.

No significant photodegradation of rhodamine WT occurred in experiments with site water. Using water exposed to bright sunlight for 1 h, concentrations of the dye were 0.49 (SD ±0.02; $n = 5$) and 0.47 (SD = ±0.02; $n = 5$) mg L^{-1} at 0- and 1-h sampling times, respectively. Earlier experiments [1] also showed no significant photodegradation of 2,4-DBE.

No significant sorption of rhodamine WT or 2,4-DBE occurred. Using site water containing 35 g L^{-1} sediment, dye concentrations were 0.52 (SD = ±0.03; $n = 5$) mg L^{-1} before and after adding sediment. Using site water containing 35 g L^{-1} leaf litter, dye concentrations were 0.52 (SD = ±0.02; $n = 5$) and 0.52 (SD = ±0.03; $n = 3$) mg L^{-1} before and after adding litter, respectively. Similar results were obtained regardless of whether or not samples were autoclaved.

Similarly, no measurable sorption occurred within 1 h using 2,4-DBE and autoclaved sediment and leaf litter. Moreover, earlier studies [1] showed that 2,4-D acid was quantitatively produced from 2,4-DBE in aufwuchs. Sorption, therefore, did not create a significant sink for 2,4-DBE. Based on chemical structure, we expected a distribution coefficient of 500 to 1,000 for 2,4-DBE. The relatively low organic composition of sandy sediment at the field site and slow penetration of 2,4-DBE into leaf litter, combined with very rapid microbial transformation occurring on all submerged surfaces, however, probably precluded any significant sorption of 2,4-DBE. Because field experiments involved similar concentrations of sediment and leaf litter and were carried out in less than 1 h under shaded conditions, we concluded that sorption and photodegradation of the dye did not significantly affect our results.

Because of the decreases in water temperature,

we expected 2,4-DBE microbial transformation rates to decrease from summer to winter. As water velocities decreased, we also expected microbial transformation rates to decrease. This expectation was based on earlier studies [1] showing that 2,4-DBE is mass-transport-limited for attached microbiota. Determinations of k_1, however, showed that transformation rates at the field site were highest (approximately three times the spring value) when water temperatures were lowest (Fig. 1). Batch cultures of mixtures of water column, sediment and leaf-litter microbiota taken from the site in February and tested in the laboratory for temperature effects showed, on the other hand, that even winter-adapted populations significantly decreased their 2,4-DBE microbial transformation rates at between 15 and 5°C (Fig. 2). Therefore, the increased transformation rates observed during the winter at the field site could not be explained as an increase in the specific activity of psychrophiles as compared with mesophilic organisms that transformed 2,4-DBE during the summer.

Furthermore, because virtually all aerobic bacterial species and all but a few fungal species typically isolated from freshwater systems can rapidly degrade 2,4-DBE [2], it is unlikely that community structural changes markedly altered transformation rates in the stream. This conclusion is also supported by data obtained in a separate study using a series of large flow-through laboratory ecosystems in which community structures varied according to nutrient inputs, yet 2,4-DBE transformation rates were relatively constant among the ecosystems [1].

A comparison of microbial transformation rate coefficients for suspended organisms to gross transformation rates for the stream showed that 2,4-DBE transformation was primarily mediated by organisms attached to surfaces. Suspended organisms accounted for only 4.6% of observed k_1 values (SD = ±3.8%; $n = 6$). In a separate study [1], laboratory experiments showed that 2,4-DBE transformation rates for attached microbiota were directly proportional to colonized surface area. Therefore, because of the predominance of attached organisms, transformation rates in the shallow stream should have been influenced greatly by colonized surface area. In visual examinations of the site from summer to winter, we noted that a rapid accumulation of leaf litter occurred in the stream during the fall and winter. The extent of leaf-litter accumulation was such that decreased water velocities recorded during the fall and winter appeared to be the result of impeded water flow through the litter. Transformation rate studies using batch samples of leaf litter showed that the litter was colonized with 2,4-DBE-transforming organisms, and that microbial transformation rates were first-order to leaf-litter concentrations of up to approximately 30 g L^{-1} (Fig. 3). Estimates of leaf-litter concentrations at the field site in February were 42 ± 14 g L^{-1}.

Based on these observations, we conclude that the winter increase in 2,4-DBE transformation rate coefficients resulted from microbial activities associated with leaf litter. Furthermore, Figure 3 indicates that k_1 values could be normalized to leaf-litter concentrations to yield a second-order rate constant only up to a concentration of approximately 30 g L^{-1}. Microbial transformation rates for Chalet Stream, therefore, may be overestimated if a second-order rate coefficient, based on leaf-litter concentrations, is used.

The increase in transformation rate coefficients did not occur until January, indicating that the leaf litter required several months for sufficient colonization to supersede the overall decreased transformation rates resulting from lower water temperatures. Other studies show that surfaces require at least 6 weeks to become maturely colonized with respect to 2,4-DBE transformation rates in warm water and take much longer in cold water [4]. It is not surprising that several months would be required for the colonization of leaf litter to overcome otherwise lower transformation rates at lower temperatures.

The significance of seasonal effects of leaf litter on microbial activities in lotic systems in temperature zones, similar to our field site, is evident in that autumn-shed leaves are considered to be perhaps the most important factor in the origin of food chains [5]. After falling into streams and rivers, litter is quickly colonized by a variety of waterborne fungi. Because of the seasonal litter fall in temperate climates, concentrations of fungal spores and other propagules in lotic systems are higher in cooler months and are minimal in summer. Exceptions include streams located in areas in which leaf fall occurs indiscriminately throughout the year [6,7]. Grazing of leaf litter by stream animals, whose main growing period is from autumn to spring, also produces large amounts of feces and causes an increase in stream bacterial concentrations [5].

In summary, 2,4-DBE transformation rates at the field site were not significantly lower in the cold months than in the summer. Furthermore,

transformation rates peaked up to several times summer rates following decreases in temperature during the fall and winter. Seasonal accumulation of leaf litter in the stream was associated with the decreases in temperature. We concluded that the additional colonized surface area provided by the litter and perhaps also by feces of grazing animals caused the increased 2,4-DBE transformation rates. Consequently, it appears that increased colonized surface area associated with leaf-litter accumulation may more than compensate for otherwise lower pollutant transformation rates in streams located in temperate wooded areas during cold seasons. Based on laboratory studies on the effects of temperature on microbial transformation rates, we did not expect these results. Therefore, the value of field studies in addressing such ecological phenomena as microbial transformation rates has been supported by this work.

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